

# Genetic and Environmental Factors Affecting Host Response to Drugs and Other Chemical Compounds in Our Environment

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Compared to laboratory animals, humans are extremely heterogeneous with respect to the many factors that can influence the distribution and biological effects of toxic chemicals. This heterogeneity can prevent an accurate assessment of the impact of a particular toxic compound on the health of an individual subject. Some of the factors that can significantly modify the host response to certain drugs, which serve in this review as a model for environmental chemicals, are enumerated and discussed. Although the mechanisms by which many of these factors modify the biological effects of certain environmental chemicals and drugs have been determined in some cases, better definition of the nature of interactions between these factors and environmental chemicals in a particular individual is required at a biochemical and molecular level. Recommendations are offered for the further development of our knowledge concerning interactions between environmental chemicals and such factors in a particular individual.

This background document considers certain aspects of the impact of environmental chemicals on the development of disease and injury in man. The primary focus is the role played by certain key variables in determining how humans respond to environmental chemicals and drugs. Since few experiments have been performed, for obvious ethical reasons, on the biological effects of exposing human populations to clearly established toxic chemicals, we are unaware at the present time of the precise way in which host variations, diet, genetic constitution, or prior disease states influence the degree and type of toxicity experienced by individuals exposed to toxic chemicals. Nor has it proved satisfactory to extrapolate from laboratory animal data to man because species differences are great with respect to the disposition of drugs and toxic chemicals. Hundreds of examples of large species differences in drug disposition have been published. Moreover, in many cases estimation of the dose that different human beings receive as a

result of chronic exposure to potentially toxic chemicals in the environment of our modern industrial cities is difficult. Effects and interactions of various potentially toxic chemicals in the environment are difficult to assess because both the chemical nature as well as the concentration of many of these environmental compounds remain to be defined precisely. For these reasons, various drugs, administered alone or in combination, will have to serve temporarily, at least, as models of how other environmental chemicals might act and interact in different individuals to produce toxicity and how certain genetic and environmental factors could modify their effects in man.

The virtue of using drugs as a model of how potentially toxic chemicals might act in man is that the chemical nature, the dose, and the route of administration of the drug can be clearly defined and the individual himself used as a control, so that relatively minor effects of various genetic and environmental factors on drug disposition can be examined. However, the utilization of drugs as a model does not by any means obviate all methodological problems. The main problem in the use of man as a subject to define environmental and genetic vari-

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ables affecting drug disposition is that man himself is exceedingly heterogeneous with respect to many genetic and environmental factors. Each human being, except for persons who are one of a set of identical twins or triplets, is unique with respect to many environmental and genetic factors that could influence drug disposition. Therefore, most studies on the role of these factors in determining drug disposition have had to be performed in experimental animals where the laboratory conditions with respect to the environment and genetic background of the animal can be most closely defined and held

investigation, may appear impressive at first glance, the precise quantitative definition of the role of each factor in determining drug disposition and the mechanisms by which each factor operates to alter drug disposition have not yet been satisfactorily elucidated in most cases. Nevertheless, the division of variables shown in Table 1 into those affecting the external environment of laboratory animals, those operating on their internal environment and those determining the pharmacokinetic and pharmacodynamic aspects by which a drug is administered may prove useful.

Table 1

Variables in the external environment	Variables in the internal environment	Pharmacologic variables
Air exchange and composition	Adjuvant arthritis	Drugs
Barometric pressure	Age	Acute vs. chronic administration
Cage design-materials	Alloxan diabetes	Bioavailability
(crowding, exercise)	Cardiovascular function	Dose, withdrawal
Cedar and other softwood bedding	Castration and hormone replacement	Presence of other drugs or food
Cleanliness	Circadian and seasonal variations	Routes of administration
Coprophagia	Dehydration	Tolerance
Diet (food and water)	Disease	Vehicle
Gravity	hepatic, renal, malignant, endocrine (thyroid, adrenal)	Volume of material injected
Hepatic microsomal enzyme induction or inhibition by insecticides, piperonyl butoxide, heavy metals, detergents, organic solvents, ammonia, vinyl chloride, aerosols containing eucalyptol, etc.	Estrous cycle	
Handling	Fever	
Humidity	Gastrointestinal function, patency and flora	
Light cycle	Genetic constitution (strain and species differences)	
Noise level	Hepatic blood flow	
Temperature	Infection	
	Malnutrition, starvation	
	Pregnancy	
	Sex	
	Shock (hemorrhagic or endotoxic)	
	Stress	

constant. Use of laboratory animals to study the factors that affect drug disposition is fraught with difficulties because the processes that control drug disposition (drug absorption, distribution, biotransformation and excretion) are exceedingly sensitive to perturbation by many diverse conditions (1).

The list shown in Table 1 (1) of genetic and environmental factors affecting drug disposition in laboratory animals is at best only partial. While this compilation, accumulated over several decades of

From what we now know of how drugs enter, pass through, and are eliminated by an organism, we can categorize certain possible mechanisms by which chemicals and drugs act and interact to produce toxicity. Table 2 identifies discrete biochemical processes and sites responsible for the disposition of drugs introduced into an organism; the variables in the external and internal environment listed in Table 1 can act to alter the disposition of a drug or toxic chemical in an organism at these sites.



Table 2

Processes	Example
Absorption of drug from its site of entry	Tetracyclines and albumin-containing antacids
Distribution of drug in the organism	Thiopental in brain and fat Warfarin displacement from albumin by various drugs, including phenylbutazone and flufenamic acid Acetylation of albumin after chronic aspirin administration, thereby altering albumin binding of other drugs
Biotransformation of drug in the organism	Phenobarbital, diphenylhydantoin, ethanol, causing induction of hepatic MFO Disulfiram and allopurinol causing inhibition of hepatic MFO
Interaction of drug with receptor sites or sites	Ethanol treatment of methanol intoxication where toxicity occurs in the organism Ethanol and chlorthimeton, barbiturates, methaqualone, etc. Nalorphine and morphine $\text{ClO}_3^-$ and $\text{SCN}^-$ as inhibitors of iodide transport into the thyroid Thioamide antithyroid drugs and peroxidase in thyroid cell $\text{H}_1$ -receptor antagonists of histamine (diphenhydramine, pyrilamine, chlorpheniramine, chlorcyclizine, promethazine) Phenoxybenzamine or dibenamine occupation of $\alpha$ -adrenergic receptor Propranolol occupation of $\beta$ receptor Acetylcholine and atropine, scopolamine Cholinergic receptor antagonism by physostigmine, neostigmine, edrophonium, DFP, paraoxon, malathion, ERN, TEPP, OMPA
Interaction of drug with elimination mechanism	Acetazolamide and carbonic anhydrase in renal tubular cell Thiazide diuretic and $\text{Na}^+$ and $\text{Cl}^-$ reabsorption in renal tubule Dose-dependent effects of benemid and salicylic acid on uric acid reabsorption and secretion by the renal tubule Heavy metal and renal tubules Renal disease and reduced renal elimination of drugs and their metabolites

## Classifying Drug Response: Susceptible Individuals and Populations

The same dose of a certain drug given by the same route to patients of the same age and sex, suffering from the same disease, may produce toxicity in some, the desired therapeutic effect in others and no effect whatever in still others. Physicians have come to recognize that in practice drugs rarely behave as ideally as they are described in most pharmacology texts and that drugs frequently do not exhibit the therapeutic effects hoped for when they are prescribed. Paul Dudley White estimated this disappointing result to have occurred in more than half his patients. The basis for the discrepancy between anticipated and actual responses to drugs lies in the immense variation among subjects. Thus, an inquiry seems warranted into the precise nature of this interindividual variation, which had previously been ill defined and dismissed with the vague term "biological variation". The same wide range of effect has been observed in the toxicity of certain chemicals, such as carbon tetrachloride or strychnine in laboratory animals (2).

nine in laboratory animals (2).

Figure 1 shows three types of curves, each describing a genetically distinct mode of inherited variation encountered clinically when the same dose of a given drug is administered by the same route to a group of patients or normal volunteers, and a particular quantitative response to a drug is measured. This measurement could be the concentration of the drug or of its metabolites in biological fluids or the change produced by the drug in the patient's pulse rate, blood pressure, or activity of a particular enzyme.

The simplest curve describing variation is the unimodal, Gaussian curve (type A in Fig. 1) (3), but its single-humped, deceptively simple curve is the most difficult to interpret. The first interpretation of the unimodal distribution curve is genetic similarity of the population tested with respect to the genes controlling the particular response being measured. For example, each of the subjects investigated may have the same genetically-determined blood group or the same activity of a specific drug-metabolizing enzyme, such as plasma pseudocholinesterase, the enzyme responsible for biotransformation of succinylcholine.



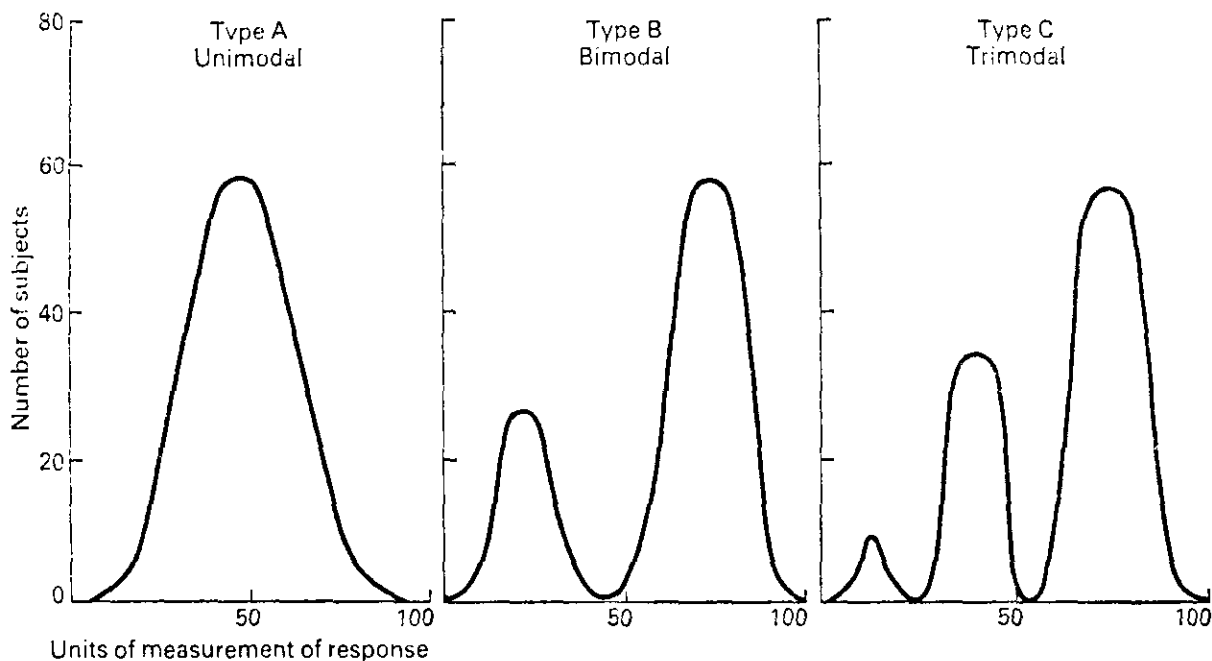


FIGURE 1. Three distinct distribution curves for response to a drug by individuals in a population. See text for an explanation of the genetic mode of transmission responsible for producing each curve.

At least two alternative interpretations of this unimodal distribution curve should be considered. The first of these questions is whether the test employed was sufficiently sensitive to separate inherently different populations from one another. Application of a more sophisticated test might possibly distinguish further among the subjects by producing multimodal curves from the unimodal curve. This situation has actually been found several times in pharmacogenetics. For example, while plasma pseudocholinesterase activity (measured in a large population with the substrate benzoylcholine) produced a broad unimodal curve, this same population studied with the pseudocholinesterase inhibitor dibucaine, as well as with benzoylcholine, yielded a trimodal distribution, like that shown as type C in Figure 1. Furthermore, family studies of atypical plasma pseudocholinesterase later proved that the mode of inheritance of this aberrant enzyme corresponded to type C, rather than type A. Thus, distinction between type A and type C is important because each curve represents a different genetic mechanism or mode of transmission. Type A, as previously noted, may arise from a population of individuals identical with respect to the genes at the genetic locus controlling the trait being measured, whereas type C represents expression of a trait inherited in an autosomal recessive fashion. Each of

the three peaks in type C corresponds to a different combination of genes at the genetic locus governing the trait being measured. Autosomal recessive inheritance indicates that affected individuals who are shown in the smallest peak in type C have a double dose of a rare mutant gene responsible for low activity of a particular enzyme or protein. The middle peak in type C corresponds to heterozygotes in the population. They possess one mutant and one normal gene at the particular genetic locus in question, and they exhibit concentrations of the involved enzyme or protein intermediate between those for affected and for normal individuals. The third and largest peak in type C represents homozygous normal individuals possessing two normal genes and thus exhibiting highest activity of the specific enzyme or protein controlled by this genetic locus. Type B represents the characteristic distribution for a trait transmitted as a Mendelian autosomal dominant. Unlike autosomal recessive traits, where two abnormal genes must be present at the genetic locus to give full expression of the trait, traits inherited in an autosomal dominant fashion require, for full expression, the presence of only a single abnormal gene at the particular genetic locus controlling the trait.

The second alternative explanation for the unimodal distribution is that the trait being measured is



controlled by genes at multiple loci, rather than the single genetic locus that gives rise to type B or type C. Genes at multiple loci play important roles in regulating such metrical, quantitative traits as stature, intelligence quotient, blood pressure, and large variations in elimination rates of certain drugs. Also, multigenetic effects have been postulated in certain congenital malformations, such as cleft lip and palate, pyloric stenosis, and clubfoot, and in certain common disorders such as peptic ulcer and diabetes mellitus.

## Single Factor Inheritance

A condition inherited as a single factor is controlled by alleles at only one genetic locus; such pharmacogenetic conditions, which are transmitted as single factors, are listed in Table 3. As opposed to inheritance patterns of traits transmitted as single factors, in polygenically controlled traits the multiple genes at several discrete loci each make a quantum contribution. Thus, the final expression of a polygenically controlled trait is the sum of the contributions from each of these multiple loci. Whereas distribution curves of response for genetic traits inherited as single factors are discontinuous, generally being either bimodal for dominant traits (type B) or trimodal for traits transmitted as autosomal recessives (type C), distribution curves for polygenically controlled traits are continuous, illustrated in large populations by bellshaped Gaussian curves for intelligence quotients and blood pressure (type A).

Several hundred pathological states inherited as simple single factors have been identified in man and intensively investigated. Much less attention has been devoted to genetic analysis of such prevalent disorders as cancer, arteriosclerosis and hypertension, which may be to some extent under polygenic control. Genetic analysis of commonly occurring polygenically controlled pathological states is technically more difficult than genetic analysis of rare conditions transmitted as simple single factors. This is because rare abnormal genes segregate simply in several generations and can easily be traced without the complication of the introduction, through marriage, of similar abnormal genes. Another reason for the absence of extensive genetic analysis of polygenically transmitted disorders is that polygenic control may be expressed only through complex interactions with etiological factors of an entirely environmental nature.

The pharmacogenetic conditions listed in Table 3 could be expanded by including several other in-born errors of metabolism associated with abnor-

mal, toxic responses to exogenously administered compounds, such as phenylketonuria, galactosemia, Willson's disease, Crigler-Najjar syndrome, and Lesch-Nyhan syndrome. Regardless of where the line of demarcation is drawn, recognition that many hereditary conditions cause dramatic alterations in responsiveness to certain drugs has important implications. Probably many additional, as yet unidentified, genetic defects result in adverse reactions to certain drugs, and the role of heredity in controlling the expression of allergic reactions due to hypersensitivity to certain drugs remains to be defined.

## Reducing Adverse Reactions

Recently, the scope of pharmacogenetics has been extended considerably beyond the conditions listed in Table 3, largely in an attempt to apply pharmacogenetic principles for analyzing and, it is hoped, for reducing the increasing number of adverse reactions to commonly prescribed drugs. The magnitude of this problem is indicated by estimates from several medical centers that one in twenty hospital admissions results from an adverse reaction to a prescribed drug. This high rate led the U.S. Task Force on Prescription Drugs to identify adverse reactions to commonly prescribed drugs as a major medical problem. The conditions listed in Table 3, because most are rare and produced toxicity after relatively few drugs, probably do not contribute substantially to the total number of serious adverse reactions. A notable exception is glucose-6-phosphate dehydrogenase deficiency, which afflicts one in ten male blacks in the United States. Also, slow inactivation of isoniazid arises in approximately 50% of the United States population. To avoid the polyneuritis that occurs from pyridoxine deficiency, mainly in certain slow acetylators when isoniazid is given alone, pyridoxine administration is recommended with isoniazid. As indicated in the following table, several other drugs, including several sulfonamides, phenelzine, procainamide, and hydralazine, are metabolized by the same enzyme that acetylates isoniazid. Therefore, slow acetylators of isoniazid also acetylate these other therapeutic agents slowly. Patients in whom a lupus-like syndrome develops during hydralazine therapy are predominantly slow isoniazid acetylators.

Before the administration of succinylcholine as a pre-anesthetic agent, typing to detect atypical serum pseudocholinesterase may prevent adverse reactions because apnea can develop for long periods in patients receiving succinylcholine if they possess atypical pseudocholinesterase. As shown



Table 3

Name of entity	Enzyme missing	Drugs
Genetic conditions probably transmitted as single factors altering the way the body acts on drugs (altered drug metabolism)		
Acatasia	Catalase in erythrocytes	Hydrogen peroxide
Slow inactivation of isoniazid	Isoniazid acetylase in liver	Isoniazid, sulfamethazine, sulfamaprine, phenelzine, dapsone, hydralazine, procainamide
Suxamethonium sensitivity or atypical pseudocholinesterase	Pseudocholinesterase in plasma	Suxamethonium or succinylcholine
Diphenylhydantoin toxicity due to deficient parahydroxylation	?Mixed function oxidase in microsomes that parahydroxylate diphenylhydantoin	Diphenylhydantoin
Bishydroxycoumarin sensitivity	?Mixed function oxidase in liver microsomes that hydroxylate bishydroxycoumarin	Bishydroxycoumarin
Acetophenetidin-induced methemoglobinemia	?Mixed function oxidase in liver microsomes that deethylate acetophenetidin	Acetophenetidin
Genetic conditions probably transmitted as single factors altering the way drugs act on the body		
Warfarin resistance	?Altered receptor or enzyme in liver with increased affinity for vitamin K	Warfarin
Glucose-6-phosphate dehydrogenase deficiency, favism or drug-induced hemolytic anemia	Glucose-6-phosphate dehydrogenase	Analgesics Acetanilide, acetylsalicylic acid, acetophenetidin (phenacetin), antipyrine, aminopyrine (Pyramidon) Sulfonamides and sulfones Sulfanilamide, sulfapyridine, N <sub>2</sub> -acetylsulfanilamide, sulfacetamide sulfisoxazole (Gantrisin), thiazosulfone, salicylazosulfapyridine (Azulfidine), sulfoxone, sulfamethoxypyridazine (Kynex) Antimalarials Primaquine, pamaquine, pentaquine, quinacrine (Atabrine) Nonsulfonamide antibacterial agents Furazolidone, nitrofurantoin (Furadantin), chloramphenicol, <i>p</i> -aminosalicylic acid Miscellaneous drugs Naphthalene, vitamin K, probenecid, trinitrotoluene, methylene blue, dimercaprol (BAL), phenylhydrazine, quinine, quinidine
Drug-sensitive hemoglobin Hemoglobin Zurich	Arginine substitution for histidine at the 63rd position of the $\beta$ -chain of hemoglobin	Sulfonamides
Hemoglobin H	Hemoglobin composed of four $\beta$ -chains	Same drugs as listed above for G-6-PD deficiency
Inability to taste phenylthiourea or phenylthiocarbamide	Unknown	Drugs containing the N-C=S groups such as phenylthiourea, methyl and propylthiouracil
Glaucoma due to abnormal response of intraocular pressure to steroids	Unknown	Corticosteroids
Malignant hyperthermia with muscular rigidity	Unknown	Various anesthetics, especially halothane
Methemoglobin reductase deficiency	Methemoglobin reductase	Same drugs as listed above for G-6-PD deficiency



in Table 3, approximately one person in 2500 has a double dose of the gene for atypical plasma pseudocholinesterase and hence is susceptible to the possibility of prolonged apnea after succinylcholine administration.

Although most of the other conditions listed in Table 3 are rare, they have not been extensively sought and may occur more frequently in certain populations. A major contribution of pharmacogenetics is to alert physicians to the possibility that several unusual drug reactions are inherited and will thus develop in some relatives of affected persons after administration of the offending therapeutic agents. In this connection, half of the siblings and half of the children of anyone who has inherited the dominantly-transmitted gene causing the rare, but frequently fatal, malignant hyperthermia with muscular rigidity will also exhibit a similar aberrant response during anesthesia with the agents listed in Table 3. Therapy of malignant hyperthermia with intravenous procaine or procainamide has been reported to produce dramatic relief of fever and rigidity.

Table 3 (3) also reveals an interesting example of a much looser association between a genetically determined drug response and a particular disease. Persons unable to taste either phenylthiocarbamide or propylthiouracil constitute approximately 30% of the population. They are at higher risk than "tasters" for development of nodular goiter or multiple thyroid adenomas. On the other hand, toxic diffuse goiter occurs more frequently among tasters than among nontasters. The conditions listed in Table 3 have been divided into those involving defective drug metabolism and those involving defective interaction of the drug with tissues, thereby producing abnormal pharmacological responses.

## Genetic Variations in Drug Clearance

A major cause of adverse reactions is drug accumulation of toxic concentrations in certain persons who, for various reasons, are unable to eliminate normal doses of a therapeutic agent as fast as most others. Large variations in the rates at which healthy, nonmedicated volunteers clear antipyrine (4), bishydroxycoumarin (5), and phenylbutazone (6) from their plasma were reported 20 years ago from Brodie's laboratory. More recently, large differences have also been shown among normal subjects in the plasma concentrations of chlorpromazine, propranolol, warfarin, phenytoin, or procainamide after a standard dose of any one of these drugs. Formerly, it was assumed that if a given drug were administered to a popula-

tion in the same dose and by the same route, all subjects would have similar blood and receptor site concentrations of the drug, and hence that all subjects would respond similarly to the therapeutic agent. In the light of these large individual differences in rates of elimination of many drugs, the principle of wide variations in the disposition of and responsiveness to therapeutic agents is now being proclaimed. This principle of pharmacological individuality has long been appreciated by practicing physicians.

## Mechanisms of Variation

The mechanism responsible for large individual variations in plasma half-lives of several commonly used drugs was recently elucidated by the application of an old technique. The twin method, introduced by Sir Francis Galton in 1875, estimates the relative contribution of environmental and genetic factors in producing a particular trait. The method, based on a comparison for any given trait of the intratwin differences for identical and fraternal twins, assumes environmental equality. For traits controlled primarily by environmental factors, intratwin differences should be of similar magnitude in identical and fraternal twins. However, for traits controlled primarily by genetic factors, there should be a difference in the magnitude of intratwin differences, since identical twins share all their genes, but fraternal twins have in common, on the average, only half of their genes. A mathematical expression for the relative control of a trait by hereditary factors is given by the following fraction, where  $V$  represents variance and FT and IT refer to fraternal twins and identical twins, respectively:

$$\frac{(V \text{ within pairs of FT}) - (V \text{ within pairs of IT})}{(V \text{ within pairs of FT})}$$

This formula gives a range of values from 0, indicating negligible hereditary and complete environmental control, to 1, indicating virtually complete hereditary influence. Appreciable environmental contributions to individual differences in drug metabolism were expected in man on the basis of investigations in laboratory animals. Numerous environmental factors, such as exposure to inducing agents, degree of health or illness and hormonal or nutritional status, are known to alter the rates at which animals metabolize certain drugs. In mice, responsiveness to a drug such as hexobarbital differs according to age, sex, litter, painful stimuli, ambient temperature, degree of crowding, time of administration and type of bedding. Such experiments imply that in man a large component in the causation of individual variations in drug



metabolism would be environmental.

Therefore, the results of genetic studies in man on phenylbutazone, antipyrine, and bishydroxycoumarin were unexpected because they indicated in healthy, nonmedicated twins almost complete hereditary and negligible environmental control. Other twin studies on halothane and nortriptyline established predominantly genetic control over large individual differences in the metabolism of these drugs. Although in healthy, nonmedicated subjects a genetic influence over large variations in drug elimination was demonstrated by these twin studies, investigations of twins fail to define unequivocally the mode of inheritance of genetically controlled variations in drug elimination.

## Mode of Inheritance

Distribution curves of the twin data do offer clues to the mode of inheritance. For isoniazid excretion in urine, twin data suggest a multimodal distribution curve indicating single-factor inheritance, later firmly established independently by family studies. In contrast to the curves for isoniazid excretion, the distribution histograms for antipyrine and nortriptyline are closer to the unimodal, continuous curves observed in polygenically controlled traits. When family studies were performed for bishydroxycoumarin (7), nortriptyline (8), and phenylbutazone (9), the results were in substantial agreement with this genetic hypothesis. The family studies revealed a significant regression of mean offspring value on midparent value, a result consistent with polygenic control. Nevertheless, before polygenic inheritance can be firmly established as the genetic mechanism for controlling interindividual variations in the basal rates of elimination of these drugs either on the basis of twin or family studies, it would be important to measure rates of production of the major metabolites of each drug, rather than simply the disappearance rate of the parent compound. Since hepatic metabolism of each drug is complex, probably involving several distinct reactions controlled by different proteins, the investigator can obtain a more direct estimate of the function of the gene controlling one particular protein by measuring the rate of production of each metabolite independently of the others. On the other hand, measuring the disappearance of a parent drug represents a more diluted approximation of gene function because such values combine the activities of several independent enzymes, and thereby reflect the function of several different genes. Attempts have been made in pharmacogenetics to follow the appearance of a drug metabolite, rather than simply the disappearance of the parent compound; examples of such work include a

twin study of the rate of production of the major halothane metabolite (10) and a twin study on the major metabolite of nortriptyline (11).

Family studies of drug elimination may be less satisfactory than twin studies for two reasons, the first being that the disposition of certain therapeutic agents changes with age and varies according to sex. Twin studies are by definition age-corrected, but the results of family studies are difficult to interpret because of the difficulty in correcting for this poorly defined change in drug disposition with age. Secondly, rates of drug metabolism have been altered either in laboratory animals or in man by such environmental constituents as caffeine, nicotine, 3-methylcholanthrene, 3-4-benzpyrene, and various insecticides. Therefore, the closer environmental similarity of children as compared to parents could be partially responsible for changes in drug-metabolizing capacity observed in family studies. The influence exerted by numerous environmental constituents on drug-metabolizing capacity may explain why, in one family study, a correlation was found between healthy, nonmedicated husbands and wives in their plasma half-lives of phenylbutazone.

## Environmental Factors and Genetic Control of Their Expression

The genetic control of large variations among healthy, nonmedicated volunteers in rates of drug elimination has several potentially useful implications. In the first place, since rates of drug elimination are genetically, rather than environmentally, controlled in healthy, nonmedicated subjects, they should be highly reproducible, stable values. This expectation has been verified for several different drugs.

Secondly, determination of drug-metabolizing capacity in an individual might be ascertained before chronic drug administration as a guide to adjusting dosage according to individual requirements, thereby helping to reduce frequent occurrences of toxicity or undertreatment encountered when the same dose of a drug is given to all subjects. One method for measuring interindividual differences in drug metabolism and for taking these interindividual variations into account in selecting an appropriate dose of drug is to ascertain drug concentrations in biological fluids. Determination of drug and metabolite concentrations in biological fluids has assumed an important role in clinical medicine. The question concerns what agents should be closely monitored in the biological fluids of patients. In general, the ideal compound for such



**Table 4.**  
**Variations in response to xenobiotics caused by genetic factors<sup>a</sup>**

Species	Response
Mouse	<p>Chloroform susceptibility</p> <p>Hexobarbital sleeping time</p> <p>Histamine sensitivity</p> <p>High insulin tolerance</p> <p>Phenothiazine protection against audiogenic seizures</p> <p>Iproniazid lethality and hepatic injury</p> <p>Ethanol preferences</p> <p><i>d</i>-Amphetamine lethality</p> <p>Chlorpromazine sensitivity</p> <p>Serotonin toxicity</p> <p>Tumorigenesis</p> <p>Aging and spontaneous cancers</p> <p>Teratogenesis</p> <p>Aryl hydrocarbon hydroxylase induction by polycyclic hydrocarbons</p> <p><math>\delta</math>-Aminolevulinic acid synthetase induction by 3,5'-dicarboxy-1,4-dihydro-2,4,6-trimethylpyridine (DDC)</p> <p>2,3,7,8-Tetrachlorodibenzo-<i>p</i>-dioxin-produced teratogenesis</p> <p>Skin ulcers caused by 7,12-dimethylbenz[a]anthracene</p> <p>Cholesterol biosynthesis</p> <p>NADPH-generating capacity</p> <p>Induction of cytochrome P<sub>450</sub> by polycyclic hydrocarbons</p> <p>Cadmium-induced testicular necrosis</p> <p>3-Methylcholanthrene-initiated tumorigenesis</p> <p>Induction of 7-ethoxycoumarin <i>O</i>-deethylase, <i>p</i>-nitroanisole <i>O</i>-demethylase, and 3-methyl-4-methylaminoazobenzene <i>N</i>-demethylase activities by polycyclic aromatic compounds</p>
Rat	<p>Thiourea toxicity</p> <p>Trichlorobutanol stimulation of ascorbic acid and glucuronic acid excretion</p> <p>Ethanol preference</p> <p>Antipyrine metabolism</p> <p>UDPG transferase deficiency (Gunn rat)</p> <p>Serotonin toxicity</p> <p>Morphine addiction</p> <p>Sensitivity to warfarin</p> <p>Aniline hydroxylation and ethylmorphine <i>N</i>-demethylation</p> <p>Aldehyde dehydrogenase induction by phenobarbital</p> <p>Cholesterol 7<math>\alpha</math>-hydroxylase induction by phenobarbital</p> <p>Serum esterases 1, 2 and 3</p> <p>Serum esterases 4 and 5</p> <p>Liver microsomal esterase</p> <p>Susceptibility to ozone toxicity</p> <p>Coumarin hydroxylase induced by phenobarbital</p> <p>Zoxazolamine paralysis time</p> <p>Phenacetin <i>O</i>-deethylase induction by polycyclic hydrocarbons</p> <p>Acetylarlyamine (2-acetylaminofluorene) <i>N</i>-hydroxylase induction by polycyclic hydrocarbons</p> <p>Lung and aryl hydrocarbon hydroxylase induction by cigarette smoke</p> <p>Acetaminophen-caused hepatic necrosis</p> <p>Shortened survival time in mice exposed to polycyclic hydrocarbons, lindane and halogenated hydrocarbons</p> <p>Induction of biphenyl-2-hydroxylase, biphenyl-4-hydroxylase and acetanilide hydroxylase activities and naphthalene-1,2,-dihydrodiol formation by polycyclic aromatic compounds</p> <p>Reduced NAD(P):menadione oxidoreductase induction by polycyclic aromatic compounds</p> <p>UDP glucuronyl transferase induction by polycyclic aromatic compounds</p>
Rabbit	<p>Bishydroxycoumarin inactivation</p> <p>Cocaine esterase</p> <p>Lactogenic effect of reserpine</p> <p>Atropine esterase</p> <p>Metabolism of nine drugs before and after phenobarbital treatment</p> <p>Slow and rapid isoniazid inactivation (<i>N</i>-acetyltransferase)</p>

<sup>a</sup>Table from Nebert and Felton (1).



an approach is one possessing a low therapeutic index but with clearly separable ineffective, therapeutic and toxic regions of drug concentration in biological fluids. The agent should be potent, act reversibly at receptor sites, and exhibit large interindividual variations in metabolism so that the same dose could conceivably yield ineffective, therapeutic and toxic blood concentrations in different subjects. Recently, Koch-Weser has suggested that for digitoxin, digoxin, phenytoin, lidocaine, lithium, nortriptyline, procainamide, propranolol, quinidine, and salicylates, whose therapeutic serum concentration he lists, determinations of blood concentration yield significant information helpful to the physician in the management of certain patients.

In experimental animals many pharmacogenetic studies have been performed that reveal how the action of various drugs or chemicals can be affected in different species by the particular genotype of that species. Variations in response to xenobiotics caused by genetic factors are listed in Table 4.

Over the past several years, interest has focused on a wide variety of environmental factors that can affect rates of drug elimination in human subjects. Because rates of drug elimination in human subjects exhibit large interindividual variations and are influenced by so many different factors, studies designed to identify specific factors must be carefully controlled in order to exclude a host of variables that could exert influence while selecting and quantitating only a single factor as an independent variable. Without adequately controlling many of the factors listed in Table 1, what appears initially to be an effect of a single factor could eventually prove to be a product of many interacting or associated factors. The task of partitioning large interindividual variations in drug elimination of exceedingly heterogeneous populations into component parts is complicated because some seemingly pure "environmental" factors such as smoking and diet are closely associated with other environmental as well as with genetic characteristics.

In 1969 a technique was introduced to overcome this major difficulty of adequately controlling human studies on factors that affect rates of drug elimination (12). This approach depended on the use of each subject as his own control (12). Antipyrine was measured in an apparently basal state. Then a single condition in the volunteer's environment was changed, such as the institution of chronic drug administration, and antipyrine pharmacokinetic measurements were repeated during and after the period of chronic drug administration. The quantitative differences between the pharmacokinetic values obtained prior to and after

chronic drug administration or some other test condition indicated to what extent the chronic drug administration or the other test condition affected hepatic antipyrine metabolism. Several precautions and assumptions had to be taken. It was necessary to determine the apparent volumes of distribution ( $aV_d$ ) of antipyrine in both the basal and perturbed states since a change in  $aV_d$ , rather than in hepatic metabolism, could produce altered values for antipyrine half-life or metabolic clearance rates. The decay of antipyrine in plasma had to be shown to be proportional to the rate of production of the major antipyrine metabolite, 4-OH antipyrine, if decay of antipyrine in plasma was to be acceptable as a measure of its metabolism. This relationship, suggested in 1950 by the work of Brodie and Axelrod (4) on the metabolic conversion in man of antipyrine to 4-OH antipyrine, has recently been confirmed in other laboratories (13, 14). Figure 2 demonstrates

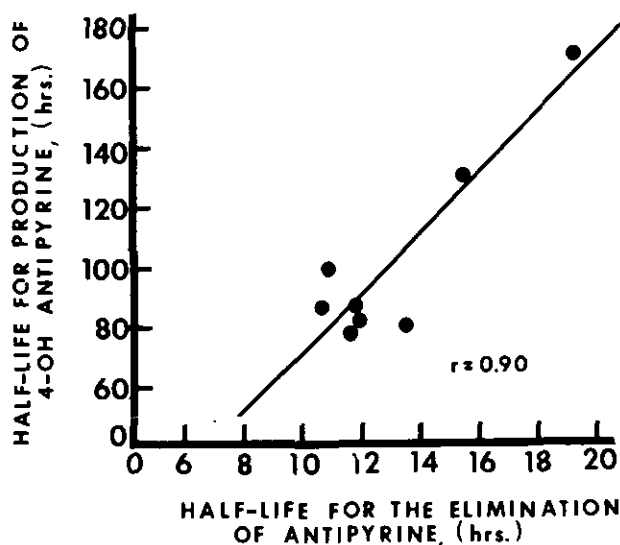


FIGURE 2. Correlation between plasma antipyrine half-life and half-life for production of 4-OH antipyrine. Reproduced from Vesell et al. (14).

this relationship for antipyrine and Figure 3 reveals that it applies as well for aminopyrine, another test drug that is now being used (13-16). The existence of a basal state of drug-metabolizing activity in a test subject needed to be investigated. Otherwise, each pharmacokinetic value obtained in a so-called "normal" volunteer, rather than being truly basal, at that time might represent a transient phase of induction or inhibition, depending on the individual's exposure to conditions or compounds that can accelerate or retard antipyrine elimination. Assurance that



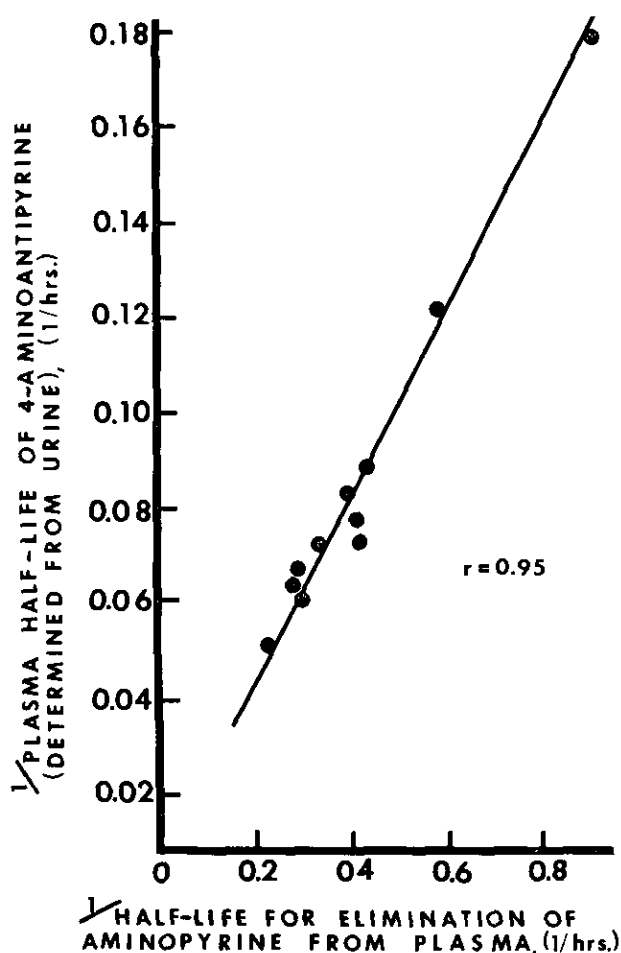


FIGURE 3. Correlation between the reciprocal of the plasma aminopyrine half-life and the reciprocal of the plasma 4-aminoantipyrine half-life calculated from 4-aminoantipyrine excretion. Reproduced from Vesell et al. (14).

an individual is in a truly basal, uninduced or uninhibited, state can never be complete; it is partially attainable through repeated measurements of the rate of elimination of a particular drug, and through a careful history of exposures at work and at home to compounds or conditions.

Table 5 provides a means of comparing the magnitude of interindividual and intraindividual variations in drug distribution. The half-lives of both antipyrine and bishydroxycoumarin are highly reproducible in a given individual; interindividual variation is approximately 10 to 20% for each drug. However, the range of interindividual variations for bishydroxycoumarin extends over 1000% and for antipyrine approximately 400%. Intraindividual variations may be attributed to environmental factors that fluctuate in their temporal impact on an individual; such fluctuating environmental factors are of small consequence compared to large, genetically controlled, interindividual variations in drug disposition (Table 5).

Although a disease process may alter the pharmacokinetic parameters of a given drug in an individual by only 20%, this negligible change can be clinically significant for a potent drug with a low therapeutic index. Such small changes in rates of drug elimination may require compensation by appropriate alterations in dose.

Determination of plasma antipyrine half-lives as a convenient, simple test of assessing rates for hepatic antipyrine metabolism (17), and its further refinement permits each individual to serve as his own control in investigating effects of drugs, environmental chemicals or other conditions on hepatic antipyrine metabolism (12). With this test, studies have been undertaken to measure the relative contributions of a number of different factors,

Table 5  
Reproducibility of plasma half-lives of bishydroxycoumarin and antipyrine in normal volunteers

Volunteer	Drug	Plasma half-life, hr		
		Initial	Repeat	% Change
D.H.	Bishydroxycoumarin	46.0	45.0	2.2
D.W.		44.0	42.5	3.4
Ge.L.		72.0	66.8	7.2
Gu.L.		69.0	70.8	2.6
Ja. T.		74.0	70.8	4.3
Jo.T.		72.0	73.6	2.2
Ja.H.		7.0	7.5	7.1
Je.H.		19.0	18.4	3.2
T.Ch.	Antipyrine	10.6	11.3	6.6
T.C.		12.5	11.4	8.8
G.Z.		7.7	7.5	2.6
T.L.		12.7	13.2	3.9
R.F.		22.4	20.5	8.5
M.R.		13.1	13.3	1.5
C.H.		9.5	10.0	5.3
P.M.		5.8	6.0	3.5



e.g., age, diet, cigarette smoking, etc., to large interindividual variations in antipyrine metabolism. While these studies can be useful in identifying qualitatively diverse factors that can potentially contribute to interindividual variations in drug disposition, pitfalls exist in quantitative interpretation of such data. For example, recent studies on factors contributing to large interindividual variations in antipyrine metabolism and in theophylline disposition claim that age, cigarette smoking, diet, and antifertility pills account for approximately half the total interindividual variation in the disposition of these drugs. Such conclusions are questionable for several reasons. One reason is that the small subpopulation examined in each study could be unrepresentative of the entire population of the particular area with respect to the incidence of the factor under examination. For example, the contribution of diet to interindividual variations in antipyrine metabolism was studied in a group receiving antipyrine in which a relatively large percentage consisted of vegetarians. However, in the total population of London, from which the study group was drawn, the number of vegetarians is exceedingly low, much less than 1%. It is erroneous to extrapolate to the whole London population the effect of diet on antipyrine disposition in their specially selected subpopulation. While in a population where 50% of the subjects are vegetarians diet may contribute significantly to large interindividual variations in antipyrine metabolism, in another very large population where there are no vegetarians, absence of meat from the diet obviously cannot be considered as contributory to the interindividual variations observed. Thus, the principle must be firmly established that quantitative extrapolation of the influence of a particular factor from one population to another is valid only if the proportion of individuals exposed to the particular factor is comparable in the two populations.

Another problem in studies of this type is encountered when different factors such as, for example, smoking and diet are examined as independent variables, regarded exclusively as "environmental." However, smoking and dietary habits are not isolated traits, but rather are inextricably connected with genetic and other environmental characteristics. Smoking habits have been correlated with intellectual, behavioral and genetic characteristics of an individual; and smoking in high school students is highly correlated with parental smoking habits (18). Thus, rather than a simple single factor of exposure to potent chemicals capable of inducing drug-metabolizing enzymes, smoking should be considered a complex trait closely associated with other environmental conditions as well as with genetic factors.

## Effects of Age and Circadian Rhythm

In most studies on drug disposition in normal male subjects living under apparently basal conditions the only limitations generally imposed on the environment were that the volunteer should not have consumed drugs for one month prior to the study; this did not constitute a major change in their habits, since they did not customarily receive drugs. In these studies, however, the influence on drug disposition of such variables as age, sex, cigarette smoking, or consumption of coffee, tea, or alcohol was not investigated. It has subsequently become clear that age plays a small role in controlling the rate of elimination of certain drugs; antipyrine half-life was 16.5% longer in older than in younger subjects (19); a poor correlation existed between age and antipyrine half-life ( $r = -0.25$ ) (19). Although changes in rates of drug metabolism with age are small compared with much larger interindividual variations of sex and age in matched normal volunteers under basal conditions, the effect of age alone on drug disposition depends on the particular drug under study. Thus, disposition of some drugs such as aminopyrine exhibit greater change with age than does antipyrine, whereas with others such as ethanol it does not change at all. Significant alterations in drug disposition occur over very much shorter periods, both circadian and seasonal changes in rates of drug disposition having been recorded. We observed that in some normal male volunteers the half-lives of both phenacetin and acetaminophen are approximately 15% shorter at 2:00 p.m. than at 6:00 a.m. (Fig. 4) (20).

Our own studies (21), as well as those of others (22), revealed that chronic ethanol ingestion increased the metabolism of drugs in some, but not all, subjects. And cigarette smoking altered phenacetin metabolism (23,24) and accelerated antipyrine metabolism (25). On an isocaloric diet, alterations of the carbohydrate to protein ratio changed antipyrine and theophylline metabolism (26). Low protein was associated with retarded metabolism of these drugs (26). Nevertheless, in obese subjects starvation for 7 to 10 days failed to alter antipyrine or tolbutamide metabolism (27). Occupational exposure to certain environmental chemicals, such as DDT, accelerated metabolism of phenylbutazone and cortisol (28), as well as of antipyrine (29). Thus some environmental factors affecting drug disposition in experimental animals (Table 1) also operate in man.

If environmental agents were mainly responsible for large variations in drug disposition among normal subjects, it would be anticipated that with in-



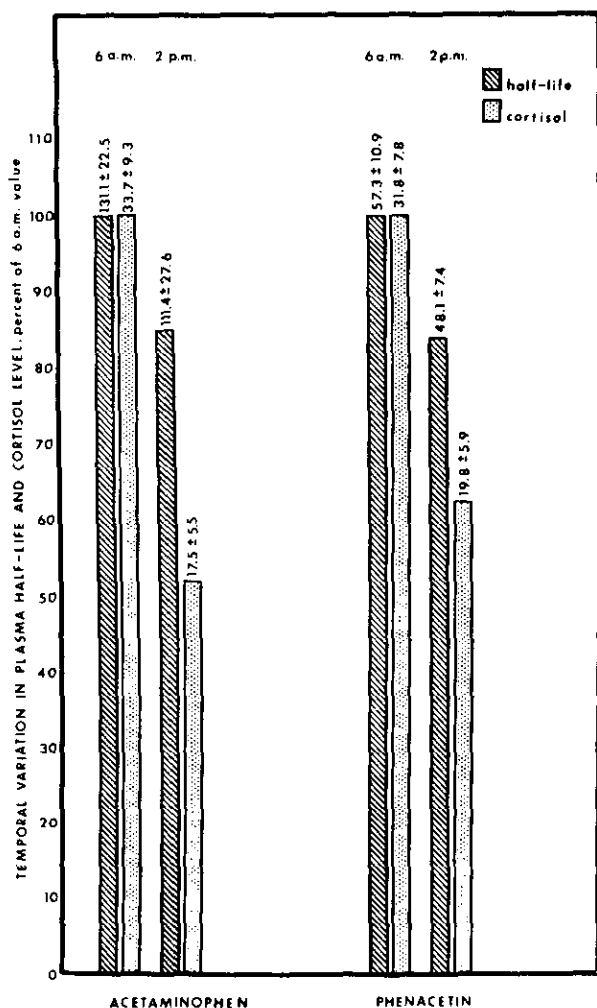


FIGURE 4. Temporal variations in mean plasma half-life (min) and mean plasma free 11-hydroxycorticoids ( $\mu\text{g}/100\text{ ml}$ ) in normal males after a single oral dose of either acetaminophen or phenacetin. Data are graphed as percent of control, using the mean 6:00 a.m. value as control. Mean  $\pm$  SD are indicated above bars. Reproduced from Shively and Vesell (27).

creasing age there would be increasing rates of drug metabolism. This supposition is based on the fact that most environmentally encountered chemicals such as DDT, PCB (polychlorinated biphenyls), and polycyclic hydrocarbons are potent inducing agents; thus, increasing exposure to these compounds with time should be accompanied by accelerated drug metabolism with age. However, interindividual variations in drug disposition cannot be explained simply on the basis of increasing exposure with time to potent inducing agents. Drug metabolism has been found to be most active in the pediatric age (30), less so in adulthood (19) and least in old age (19). Clearly other factors must be in-

involved. One commonly encountered environmental chemical, the birth control pill, inhibits hepatic drug metabolism (31, 32), but age as a factor in the observed decrease in rates of drug metabolism of this chemical can be excluded for several reasons, among them that the main age effects are observed in the pediatric and geriatric groups and that decreased drug elimination occurs with age in groups composed entirely of males.

## Effects of Disease on Drug Metabolism

Only recently was abnormal drug metabolism demonstrated convincingly to accompany various disease states. This association remained unproven so long because of the contrasting problems of investigating disease states in laboratory animals and human beings. In experimental animals the effect of disease states on drug disposition can be carefully measured because the environmental and genetic characteristics can be determined and controlled rigorously; several such disease states in which appropriate studies on animals showed that drug disposition was altered are listed in Table 1. Development of a highly reproducible model in the animal permitted precise quantitation of the effects on drug disposition produced by a specific pathologic lesion of known intensity and severity, and made possible a comparison with drug elimination under normal basal conditions in the same animal.

By contrast, in human disease each patient represents an almost unique constellation of genetic and environmental variables. Thus, variation in drug disposition due to these factors is immense. Furthermore, a very broad spectrum exists in the severity, intensity, and duration of most human disease; large changes in the disease process and its effects occur commonly with time, even when only a single organ is affected. Generally, however, several organs are simultaneously involved. The functional status of the cardiovascular, endocrine, hepatic, hormonal, and nervous systems fluctuates during many disease processes that produce time fluctuations in drug disposition. In addition, such variables as age, sex, genetic constitution, diet, weight, body size, exposure to other drugs, environmental chemicals, etc., differ among patients. In addition to these complications, the normal rate of drug elimination prior to disease is rarely known. Thus, it is often impossible to determine whether the rate of drug elimination measured in a given patient constitutes a change from his normal basal rate.

Many different disorders and pathologic states can alter the normal rates and pathways for drug



absorption, distribution, biotransformation, excretion, interaction with receptor sites or various combinations of these. Since each drug has a distinct profile for these five processes, the extent to which a disease that affects these processes will alter the distribution of a drug depends on the particular drug. Therefore, it is hazardous to extrapolate how pathologic processes will affect other drugs on the basis of pathologic effects that alter the distribution of the one drug. Furthermore, the clinical consequences of a change in drug distribution produced by disease will be determined also by the therapeutic index of the drug as well as by certain genetic and environmental characteristics of the patient. For example, a change of 200% in the plasma half-life of antipyrine, salicylates, or penicillin will probably have little or no clinical consequence, whereas a change of 20% in the plasma half-life of digoxin, procainamide, or lidocaine may prove critical. By the same token, a change of 1% in the albumin binding of warfarin, which normally is 99% bound, may have profound toxicologic results, whereas a change of 1% in the albumin binding of probenecid, which normally is 75% bound, has negligible clinical consequences. Here the crucial factor is the percent change in the free, but not bound, portion of the drug.

Effects of disease states on the rate of absorption of the drug depend on many factors including the site of drug administration. If the drug is administered orally, the influence of disease on rates of drug absorption will depend on the nature of the disease process, whether it affects the areas in the gut where the drug is normally absorbed, how the disease alters the normal physiologic volume, pH, temperature, viscosity, surface tension and composition of the gastrointestinal secretions and contents (33). Rates of drug absorption may also be influenced by whether or not food is present, the nature and quantity of bile salts and bacterial flora, the rate of splanchnic blood flow, prior diet and food intake as well as gastrointestinal motility (33). Until recently little was known about how disease states altered these factors in man; however, large interindividual differences in rates of absorption of many orally administered drugs occur in hospital patients (34-36) as well as in normal volunteers (37-39). For example, a 7-fold range in the amount of tetracycline absorbed was reported in six fasting, healthy subjects (40). Variations in gastric emptying may contribute significantly to large interindividual differences in drug absorption rate because numerous physiologic conditions, such as posture and autonomic activity, as well as the temperature, volume, viscosity and tonicity of gastric contents, can change gastric emptying time. Grossly impaired

absorption of paracetamol occurs in patients with delayed gastric emptying and pyloric stenosis (41). In patients with slow gastric emptying L-dopa may be ineffective (42). Therapeutic failure of orally administered drugs usually accompanies gastric stasis (41, 43, 44). In patients with achlorhydria, aspirin was absorbed significantly faster, and plasma salicylate concentrations were higher than in controls (Fig. 5) (44). Prescott (45) reported the interesting observation that acetaminophen plasma concentrations were significantly higher after oral administration in 12 convalescent hospital patients in bed than in seven healthy ambulant volunteers matched for age and sex.

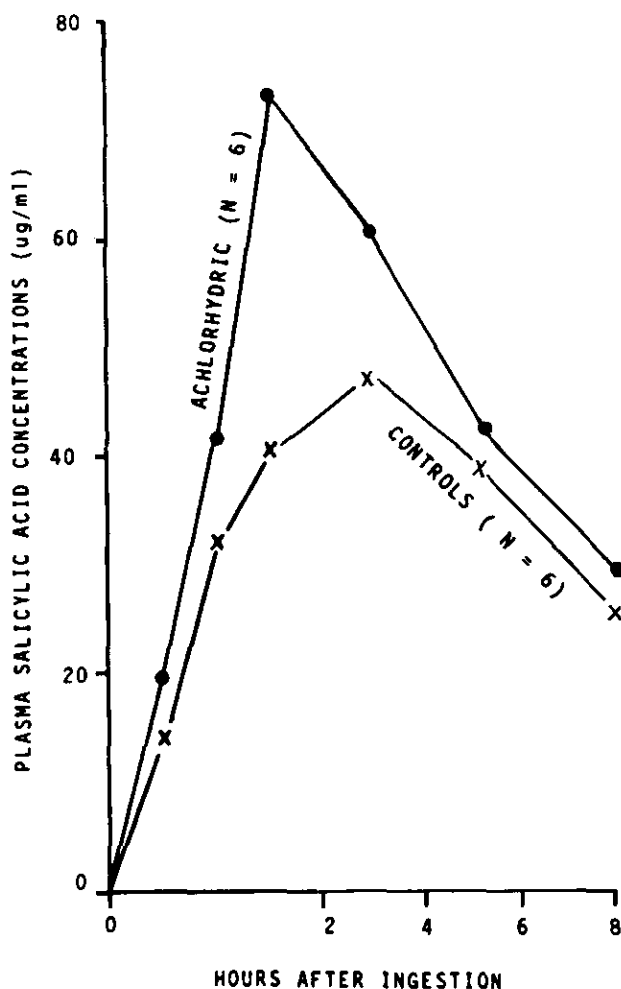


FIGURE 5. Mean plasma salicylate concentrations in achlorhydric and control patients following an oral dose of 900 mg of acetylsalicylic acid. Reproduced from Prescott (45).



Since rates of absorption of orally administered pills and capsules are dependent on rates of their dissolution and dispersion, some of the factors enumerated above can alter such rates, thereby contributing to variations in drug absorption. It is interesting and somewhat surprising that the absorption of *p*-aminosalicylic acid and insoniazid was unchanged by gastrectomy performed for peptic ulcer, although complete failure of ethionamide absorption occurred in some patients (46). Furthermore, gastrectomy failed to alter the absorption of sulfisoxazole, quinidine or ethambutol unless vagotomy had been performed, thereby slowing gastric emptying (47).

In jejunal disease folic acid absorption is diminished (48). In ileal disease the transport of bile acids may be impaired, as well as the enterohepatic transport of many lipid soluble drugs, since bile acids promote the gastrointestinal absorption of fat and certain fat soluble compounds including many drugs and vitamins A, D, K, and E. Ileal disease may be associated with impaired B<sub>12</sub> absorption, since B<sub>12</sub> is absorbed in the ileum after it forms a complex with intrinsic factor produced by the gastric parietal cell. Defective function of the ileum secondary to surgical removal or disease through interference with B<sub>12</sub> absorption can lead to pernicious anemia. B<sub>12</sub> absorption can also be impaired in gastric diseases where parietal cell function is abnormal, producing intrinsic factor deficiency and pernicious anemia. In some patients with pernicious anemia precipitating or blocking antibodies to intrinsic factor have been identified (49, 50). In addition, regional enteritis as well as tropical sprue, celiac disease, and Whipple's disease can produce B<sub>12</sub> malabsorption. In steatorrhea, fat-soluble drugs and vitamins may be lost in the feces, producing deficiency of the fat-soluble vitamins, a deficiency of vitamin D being by far the most significant chronic problem in gastrointestinal disease.

Some active drugs are produced after metabolism of the inactive form by gut bacteria, the best example being cleavage of salicylazosulfapyridine, the drug of choice in the treatment of chronic ulcerative colitis. Diseases or drugs that change the nature of the gastrointestinal flora can affect the disposition of other drugs metabolized by gut bacteria. Possibly the effect of large doses of charcoal on gastrointestinal absorption of phenacetin is mediated by gut bacteria and induction of AHH activity in these bacteria by charcoal (51). The absorption of digoxin is reduced by neomycin administration (52).

The binding of many drugs to albumin is altered in several disease states. For example, the phenytoin binding was decreased in plasma of 15 uremic patients (53). The size of the unbound fraction cor-

related well with blood urea nitrogen, serum creatinine and clinical state of the patient. In addition to phenytoin, other organic acids including congo red, sulfonamides, thyroxine, tryptophan, clofibrate, fluorescein, and methyl red exhibited decreased protein binding in uremia. By contrast, most organic bases bind normally to plasma from uremic patients. A clinically significant neutral compound, digitoxin, exhibited decreased plasma binding in uremic patients (54). Disease states including cirrhosis and nephritis are associated with hypoproteinemia and hypoalbuminemia. In both these conditions the unbound fraction of most drugs is elevated compared to the unbound fraction that exists with normal protein and albumin concentrations. These situations illustrate the danger of selecting drug dose solely on the basis of total drug concentrations in plasma, rather than on the free concentration, since only the free form is pharmacologically active. In cirrhosis, the plasma binding of the organic bases quinidine, diazepam and triamterene, as well as of the organic acid fluorescein are all decreased.

Since poor renal function is associated with decreased excretion of several drugs, it is important to modify normal drug dosage in uremic patients. Methods for this have been presented by Dettli and others (55-58). Dettli (57) described a linear relationship between the overall elimination rate constant  $k_e$  and the endogenous creatinine clearance ( $V_{cr}'$ ):

$$k_e = k_{nr} + \delta V_{cr}'$$

where  $k_{nr}$  is the mean extrarenal elimination rate constant in anuric patients and  $\delta$  is a constant relating  $V_{cr}'$  to the renal elimination rate constant  $k_r$  of the drug. This equation can be used for about 40 drugs; simple nomograms have been devised which allow the estimation of the rate of drug elimination in the patient with kidney disease from the value of  $V_{cr}'$  (57, 59). However, there are numerous drugs whose apparent elimination rates are unaltered by uremia, including tolbutamide, phenobarbital, histamine, phenacetin, phenytoin, antipyrine, quinidine, propranolol, and vitamin D (53).

Much less study has been devoted to effects of disease states on receptor sites and the kinetics of formation and dissociation of drug receptor complexes. Comparison of the basal state of receptors under normal and disease conditions should facilitate analysis of drug action. Advances in receptor isolation by techniques such as affinity labeling now permit careful *in vitro* studies to assess how diseases affect receptors. For example, such techniques have the potential of revealing whether cholinergic receptors from myasthenic skeletal



muscle function normally in response to transmitter and in the kinetics of drug binding and dissociation. Diseases can also alter the local environment of receptors and change their conformation. A classic example of how environmental conditions alter the binding of a drug to its receptor is that of digoxin with receptors in cardiac muscle, where the efficacy of the drug in producing inotropic and chronotropic effects can be appreciably altered by changes in potassium ion concentration or pH near the receptor.

## Effect of Fever on Drug Disposition

In the course of many diseases, fever, a concomitant of many diseases, develops either acutely or chronically. Yet, until recently, the effects of fever on drug distribution were unexamined. In 1975, it was shown that etiocholanolone-induced fever in normal volunteers was associated with the prolongation of plasma antipyrine half-life in 11 of 14 volunteers who developed fever (60) (Fig. 6). These results have been confirmed by Trenholme et al. (61), who demonstrated retardation of quinine elimination both in etiocholanolone-induced fever and in fever associated with experimentally induced malaria. The effects of drug distribution need to be studied both with more drugs and in other febrile states before generalizations concerning the role of fever in drug disposition and appropriate dosage compensation can be made.

## Effect of Thyroid Disease on Drug Disposition

An extensive literature based on experimental animals describes various effects produced by altered thyroid function on drug disposition. In 1973, patients with hyperthyroidism were shown to exhibit accelerated antipyrine metabolism, whereas patients with hypothyroidism displayed prolongation of plasma antipyrine half-life (62) (Fig. 7). These observations were confirmed in 1974 (63) and were extended in 1975 (64) (Fig. 8) to other drugs. Thus, for drugs of low therapeutic index that are metabolized by the hepatic mixed function oxidases, the appropriate dose can be significantly altered by the functional status of the thyroid.

## Studies on Drug Distribution in Patients with Liver Disease

For many years uncertainty prevailed concerning

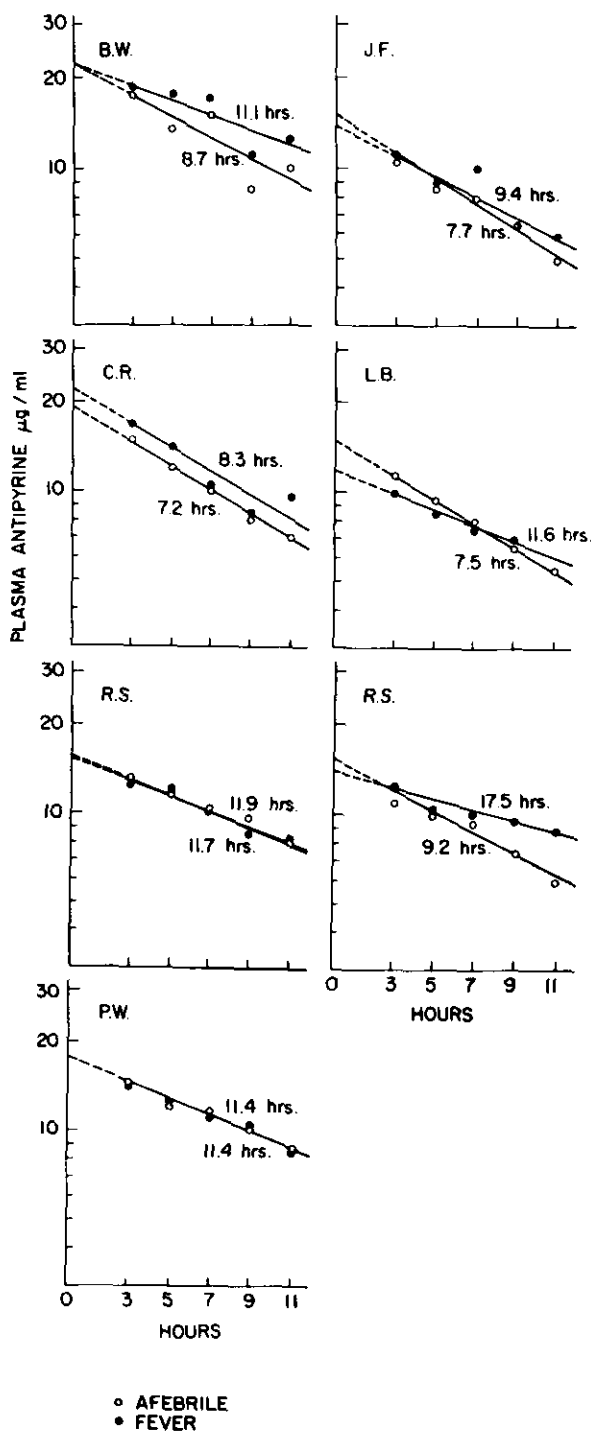
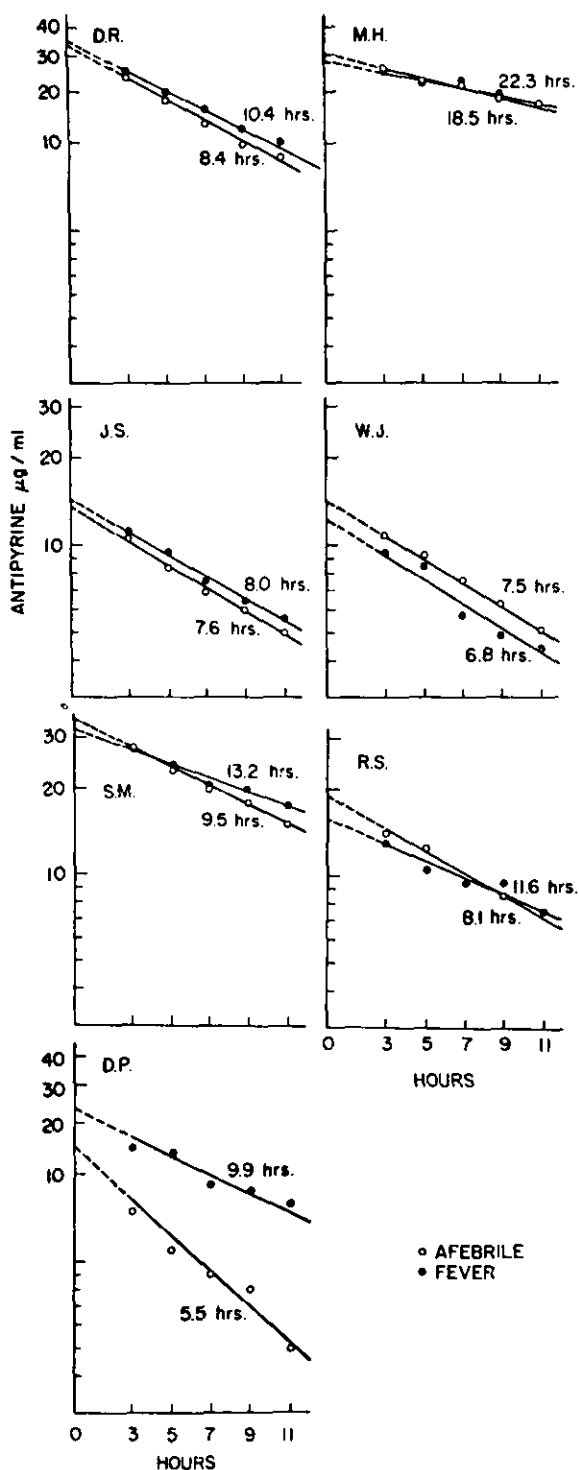


FIGURE 6. Plasma antipyrine half-life after an oral dose of 10 mg/kg antipyrine in aqueous solution given to normal volunteers in an afebrile state (○) and again during production of fever by etiocholanolone administration (●). Note prolongation of plasma antipyrine half-life by fever in most, but not all, volunteers. Data from Elin et al. (61).





the relationship between liver disease and drug metabolism. Levi, et al. (65) in 1968 revealed that this ambiguity arose in part from failure to consider how concomitantly administered drugs affected the metabolism of the drug under study. In 1971 Cooksley and Powell (66) showed that the metabolic pathway for the hepatic biotransformation of prednisone was affected by liver disease and that the extent of this effect depended on the nature and severity of the disease. In 1972 Faigle (67) reported that in patients with severe bilharziasis, metabolism of the schistosomacide niridazole (nitrothiamidazol) was impaired, as revealed by higher blood levels of the drug and a higher incidence of adverse effects than in normal subjects. In 1973 Branch et al. (68) reported that serum antipyrine half-life was prolonged in patients with liver disease, an observation confirmed in 1974 by Andreasen et al. (69) and by Andreasen and Vesell (70) (Fig. 9). Also in 1974, Hepner and Vesell (15) reported that aminopyrine metabolism was deranged in a large percentage of patients with liver disease and proposed an aminopyrine breath test (ABT), in which  $^{14}\text{CO}_2$  was measured in breath after the administration of 4-dimethyl- $^{14}\text{C}$ -aminopyrine ( $^{14}\text{C}$ -aminopyrine), as a sensitive, clinically convenient test of hepatic function and drug metabolism. The virtue of this method was that it measured production of a metabolite, rather than disappearance of the parent drug. It is now abundantly clear from numerous studies that the rate of disappearance of cold aminopyrine or antipyrine in plasma of a patient correlates highly with the rate of production of their major metabolites (14).

Further studies utilizing patients with various hepatic disorders disclosed that the ABT differentiated different types of hepatic dysfunction (71) (Fig. 10). For example, in 16 of 21 subjects with cholestasis not caused by malignant disease, the mean  $^{14}\text{CO}_2$  excretion was normal. The mean percentage of administered  $^{14}\text{C}$  excreted in  $^{14}\text{CO}_2$  in 2 hr in control patients was  $7.1 \pm 1.3$  (SD)% and significantly less ( $p < 0.01$ ) in patients with portal cirrhosis ( $2.6 \pm 1.2\%$ ), fatty liver ( $4.7 \pm 1.1\%$ ), hepatitis ( $2.6 \pm 1.4\%$ ), and hepatic malignancy ( $3.5 \pm 1.8\%$ ) (71) (Fig. 10).

It is now firmly established that hepatocellular disease is associated with decreased capacity to metabolize some drugs. However, it has not yet been determined whether the extent of depression of hepatic drug-metabolizing function in an individual with liver disease is similar for all drugs biotransformed by the liver.

Our observations that rates of aminopyrine elimination are reduced in almost all patients with parenchymal liver disease and observations by



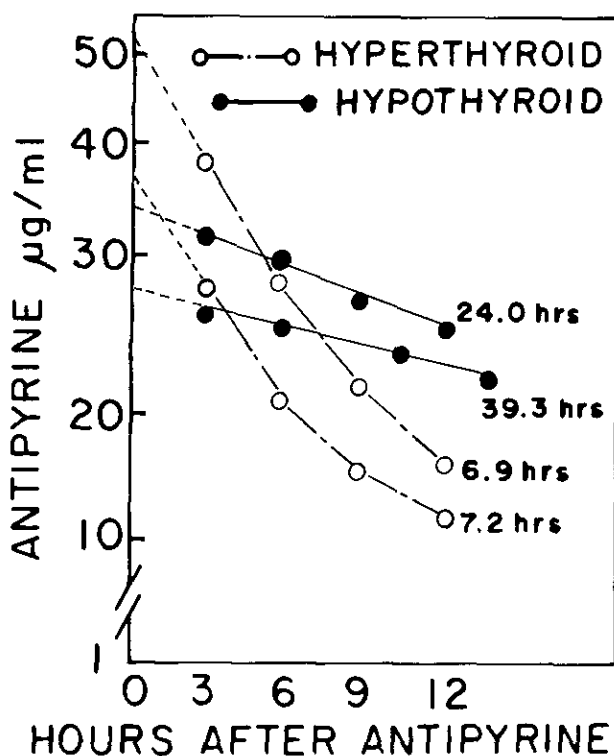


FIGURE 7. Prolonged antipyrine half-lives in two hypothyroid patients and shortened antipyrine half-lives in two hyperthyroid patients relative to the normal antipyrine half-lives in 36 healthy volunteers of  $10.9 \pm 0.5$  hours. Reproduced from Vesell and Passananti (63).

Branch (68) and Andreassen (69, 70) that liver disease is also accompanied by reduction in rates of hepatic antipyrine metabolism can be harmonized with observations of Williams et al. (72) and that warfarin disposition during acute viral hepatitis is unchanged and of Shull et al. (73) that oxazepam disposition is normal during acute viral hepatitis and cirrhosis. Between these extremes, a group of drugs of which clindamycin is an example apparently produce intermediate or moderate changes in disposition in liver disease (74). A wide range, from no change whatever in disposition to significant retardation, has been reported in liver disease, depending on the drug studied. For drugs with high hepatic extraction ratios, greater than 0.8, such as propranolol and lidocaine, alterations in blood flow accompanying liver disease can produce large changes in hepatic clearance of the compound. For drugs with very low hepatic extraction ratios, less than 0.2, such as antipyrine and aminopyrine, large variations in the extent to which hepatocellular disease alters their rates of metabolism cannot be due to abnormal liver blood flow. They may be attributed in part to multiple molecular forms of hepatic

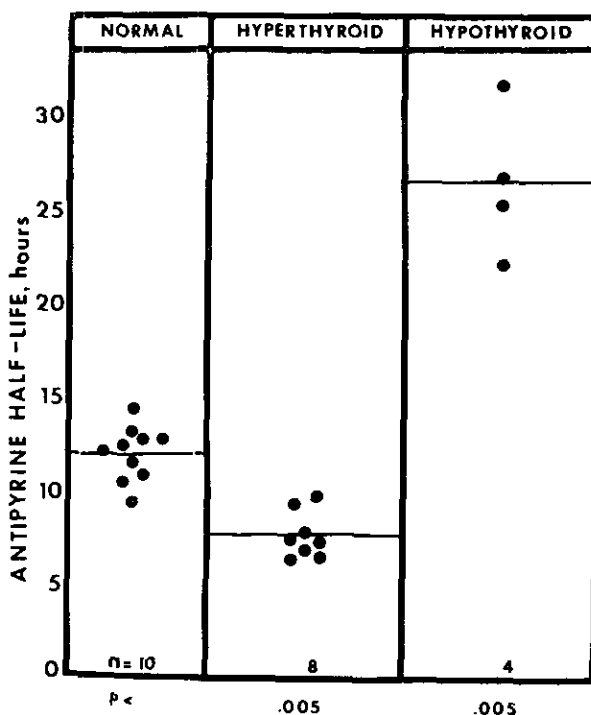


FIGURE 8. Values for plasma antipyrine half-life in normal subjects and in patients with hyperthyroidism and hypothyroidism. Each closed circle represents the value for a single individual. Reproduced from Vesell et al. (65).

cytochrome P-450 and to the differential effects that a particular hepatic disorder might exert on these forms.

Heterogeneous effects of liver disease on drug disposition are in accord with previous studies in normal individuals that established lack of correlation in rates of hepatic biotransformation of different drugs. In normal individuals rates of hepatic biotransformation of antipyrine did not correlate with rates of hepatic biotransformation of such other drugs as phenylbutazone and bishydroxycoumarin (75), an observation confirmed by Kadar et al. (76). No inherent incompatibility exists between failure to correlate rates of drug metabolism in the basal state and high correlation of these rates when the hepatic drug-metabolizing system is induced or inhibited. One explanation for this is the heterogeneity, both qualitative and quantitative, of cytochrome P-450, each of the multiple forms probably acting on different drug substrates (77). When an inducing or inhibiting compound is administered, one (or a few) of these forms is selectively altered. Consequently, a greater uniformity of response occurs in different subjects as identified by adminis-



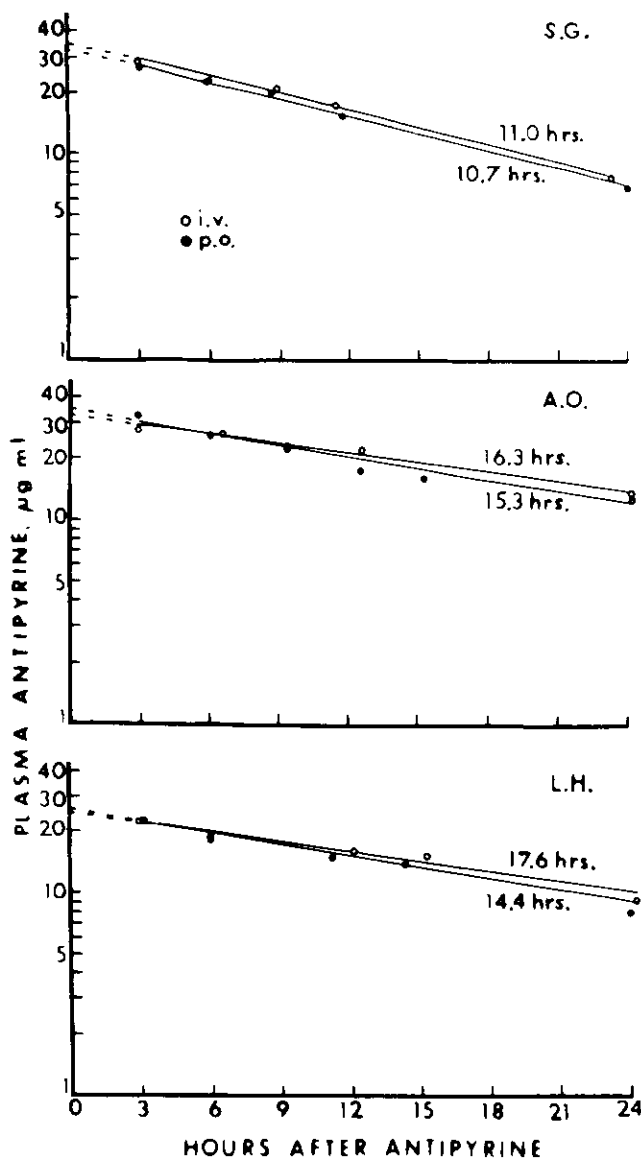


FIGURE 9. Decay of antipyrine after oral and intravenous administration to three patients with cirrhosis of the liver. The oral and intravenous doses were separated by an interval of 8 days. Reproduced from Andreasen and Vesell (71).

tration of a test drug if that drug is the substrate for the particular cytochrome P-450 that has been altered. Although large interindividual variations in the extent of induction as measured by percent change in plasma antipyrine half-life from the basal level have been reported (12), an inducing agent such as phenobarbital reduced interindividual variations in antipyrine elimination rates measured in unmedicated, normal volunteers. A more uniform

rate of antipyrine metabolism was produced by phenobarbital for all subjects in the study, even though in some it changed more than in others to attain this rate (12). These observations are compatible with and help explain lack of significant correlations among rates of elimination of some drugs in the basal, unmedicated state, but significant correlation in the induced state of drug metabolism.

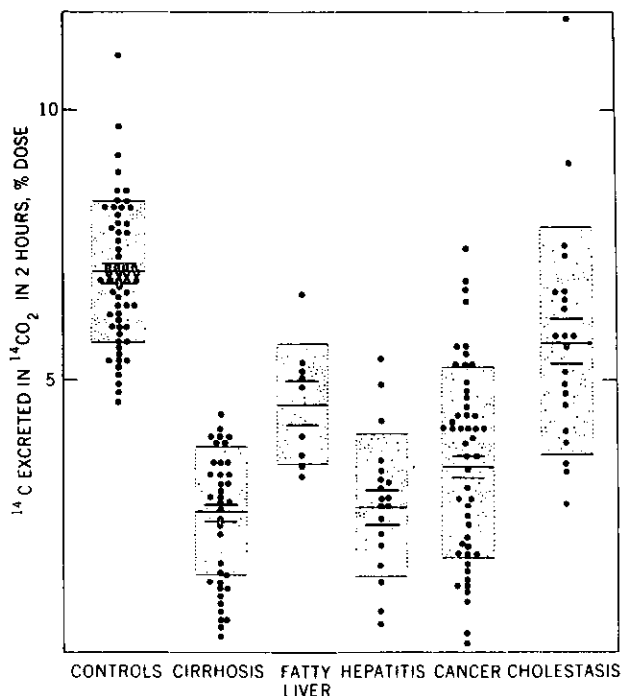


FIGURE 10. Percentage of administered  $^{14}\text{C}$  excreted as  $^{14}\text{CO}_2$  in breath 2 hours after oral administration of  $[^{14}\text{C}]$ aminopyrine. Transverse lines represent mean  $\pm$  SEM; hatched areas represent SD. Reproduced from Hepner and Vesell (72).

## Methodologic Problems

### Predictive Values of Correlation Coefficients Between Rates of Elimination of Different Drugs in the Same Subject

A discussion of the biologic significance of correlations among pharmacokinetic values of several drugs determined within the same normal volunteer seems warranted because of the current popularity



of such correlations and the misconceptions concerning their clinical applicability. The principal aim of such correlations among rates of elimination of different drugs in the same subject is to predict in a given patient the behavior of one drug as against another. The predictive value is not equivalent to the correlation coefficient, as implied in so many recent studies, but rather to the square of the correlation coefficient. In clinical practice, the usefulness of predictive values of less than 90% is limited; to achieve a predictive value of 90%, the correlation coefficient must be 0.95. With lower correlation coefficients, the difference between the predictive value and the correlation coefficient is larger. For example, a correlation coefficient of 0.80 has a predictive value of 0.64; and a correlation coefficient of 0.60 has a predictive value of 0.36, so that in only 1 of 3 patients would a prediction based on such a relationship be accurate. It must be stressed that a low  $p$  value does not necessarily indicate that the correlation is biologically significant or clinically useful. The  $p$  value does not provide information on the biologic significance of a relationship, but rather on the probability of reproducing the best line that can be drawn through as many of the data points as possible. Thus, a correlation coefficient of 0.30 or 0.60 or even 0.80 may have a  $p$  value of 0.001, but the relationship between the rates of elimination of the drugs is too poor for practical clinical application, since even the correlation coefficient of 0.80 yields a correct prediction in only 2 of 3 patients. Furthermore, studies on small numbers of subjects may yield a very high correlation between the elimination rates of several drugs; such relationships should be rechecked with larger groups, since some high correlations obtained with small numbers of volunteers have not been reproducible, even in the same laboratory (78). Present data suggest that the extent to which hepatic dysfunction alters rates of elimination of exogenous compounds removed by the liver varies according to the substrate. Therefore, when patients with hepatic dysfunction receive drugs eliminated through hepatic mechanisms, the rate of elimination of one drug may not be accurately extrapolated from pharmacokinetic information concerning another; future work may reveal discrete categories of drugs so that how one drug in a category is eliminated in a patient with liver disease will accurately enable extrapolation to other drugs in the same category. In principle, however, the results in patients with liver disease agree with observations in normal, uninduced volunteers (79), where one test drug does not satisfactorily provide information concerning the disposition of others.

## **Hazards of Genetic Analysis Based on Distribution Curves**

Finally, there are limitations and pitfalls inherent in interpreting distribution curves of drug response. It is becoming increasingly popular to construct distribution curves to investigate the quantitative variations in the elimination rate of or response to the same dose of a drug administered by the same route to a group of unrelated normal subjects. Such curves appear with increasing frequency in the literature, since this approach apparently permits some degree of genetic analysis in groups of normal, unrelated volunteers, despite extreme genetic and environmental heterogeneity with respect to the factors (Table 1) that can affect drug elimination and response. A bimodal or trimodal distribution curve obtained in such groups is generally interpreted to indicate that interindividual variations in drug elimination or response are caused by genes at a single locus (monogenic control), whereas a unimodal distribution curve suggests control by genes located at multiple loci (polygenic control). It must be emphasized that by itself the shape of the distribution curve cannot prove or disprove a genetic hypothesis: the only acceptable method of establishing the mode of inheritance of a trait in man is to study its transmission in families. Distribution curves can be helpful as clues on which to base family studies, but can be misleading when relied on without family studies. For example, unimodal or multimodal distribution curves can arise entirely from environmentally controlled factors or can be generated by variations controlled entirely by genes located either at multiple loci or at a single locus. Thus, distribution curves as currently used in pharmacogenetics cannot differentiate among these alternatives. Moreover, it is often impossible to determine the precise shape of a distribution curve for a small number of subjects. Discontinuity in the distribution curve for unmetabolized isoniazid in the 24 hr urine collected from ten sets of twins is demonstrated in Figure 11. Trimodality was later firmly established by appropriate family studies that demonstrated autosomal recessive inheritance of interindividual differences in the rates of isoniazid acetylation. However, the distribution curves for antipyrine and nortriptyline are less clear from the few individuals shown in Figure 11. While the distribution of antipyrine half-lives in the plasma of 14 sets of twins could be compatible with trimodality, the distribution might also be unimodal. Studies on larger numbers of subjects in several different laboratories yielded a unimodal distribution curve for antipyrine half-lives or metabolic clearance



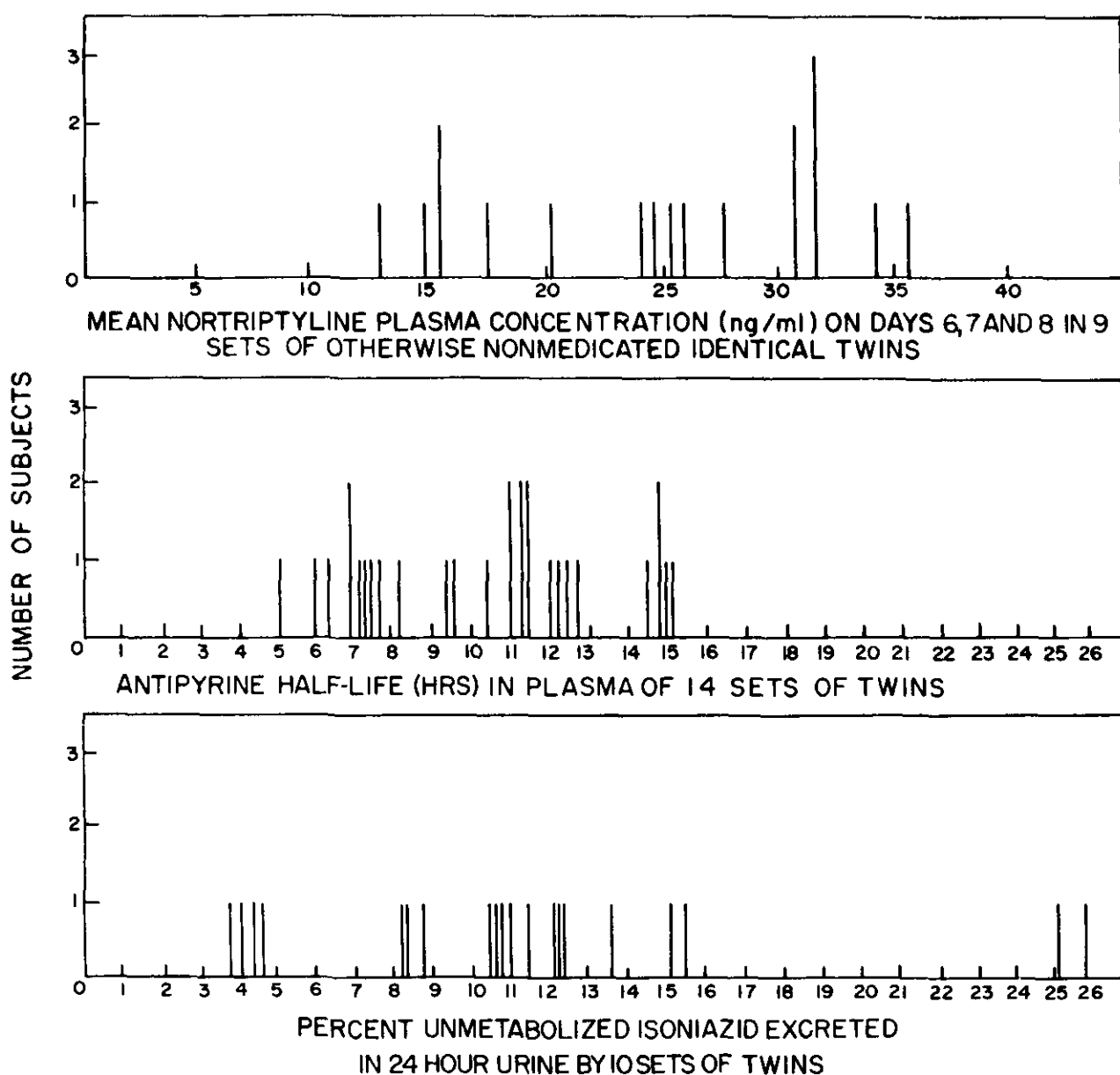


FIGURE 11. Distribution curves of response in three twin studies. Although the distribution is clearly multimodal for 24 hour excretion of isoniazid, the distribution of antipyrine half-lives in plasma and for steady state blood levels of nortriptyline is less clear cut, the curves being compatible either with a unimodal or polymodal interpretation.

rates.

Plotting distribution curves based on pharmacokinetic data may not accurately reflect rates of drug metabolism, since many diverse factors influenced by various genetic as well as environmental characteristics affect such pharmacokinetic parameters as the plasma half-life or even the metabolic clearance rate of a drug. Thus, for many drugs, distribution curves utilizing purely pharmacokinetic data can be misleading because genetic

interpretations of these distribution curves suggest that the trait being measured is much closer than it actually is to a single gene product. This limitation applies as well to family studies.

## Future Needs and Directions of Inquiry

This review of the present state of knowledge



concerning the role played by certain key variables in determining how humans respond to environmental chemicals and drugs has identified several future needs and directions of inquiry.

In order to define the impact of various potentially toxic chemicals in the environment on human populations, it is necessary to estimate more accurately the specific concentrations of various toxic chemicals in different geographical areas and under different working conditions, as well as to determine whether such potentially toxic compounds actually are present in human biological fluids and tissues.

The normal mechanisms by which these toxic compounds are handled must be better defined in man and here, too, the need for better knowledge of how to extrapolate from animal studies to man is obvious.

Improvements in methodology are required at various levels: (a) The further development of chemical analytical methods and kinetic techniques for the recognition of trace amounts of chemicals as well as for the development of substitute surrogate or model compounds where it would be unethical to study highly toxic compounds in man; (b) the application of more refined genetic analyses to polygenic systems; (c) the development of methods to identify the discrete components of polygenic systems by laboratory techniques; (d) the use of *in vitro* techniques (including tissue culture) to predict the response of individuals to drugs or compounds in the environment.

The study of population genetics in order to: (a) identify particular populations at high risk because of their possession of certain alleles in high frequency and (b) to elucidate ethnic variability in the metabolism of or response to drugs and toxic compounds.

The pathophysiological mechanisms underlying proven associations between polymorphism and disease, especially those produced or enhanced by drugs, must be elucidated.

Genetic factors should be looked for when attempts are made to unravel the mechanisms of clinically significant drug interactions.

It is critical to develop adequate animal models to study the problems of human exposure to toxic chemicals. We need to define better how far we can safely extrapolate data from laboratory animals to man if laboratory animals are to serve as satisfactory models for the issues we are addressing.

In searching for genetic factors that cause interindividual differences in response to environmental chemicals and drugs, there is a need to measure rates of production of the major metabolites of a toxic compound or drug, rather than the rate of disappearance of the parent chemical.

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